REMARKS

Rejection of Claims and Traversal Thereof

In the January 2, 2003 Office Action,

claims 14-25, 27, 29, 38-61, 65 and 66 were rejected under 35 U.S.C. §112, second paragraph;

claims 14-25, 27, 29, 38-61, 65 and 66 stand rejected under 35 U.S.C. §103 (a) as being unpatentable over Donnelly, et al. (WO 96/00583) and Johnson (U.S. Patent No. 5,658,785) in further view of Whittle, et al. (U.S. Patent No. 5,658,785);

claims 16, 18, 20 and 50 stand rejected under 35 U.S.C. §103 (a) as being unpatentable over Donnelly, et al. (WO 96/00583), Johnson (U.S. Patent No. 5,658,785) and Whittle, et al. (U.S. Patent No. 5,658,785) in further view of Gissmann, et al. (WO 96/11272); and

claim 61 stands rejected under 35 U.S.C. §103 (a) as being unpatentable over Donnelly, et al. (WO 96/00583), Johnson (U.S. Patent No. 5,658,785) and Whittle, et al. (U.S. Patent No. 5,658,785) in further view of Stanley, et al. (U.S. Patent No. 6,096,869).

These rejections are hereby traversed and reconsideration of the patentability of the pending claims is requested in light of the following remarks.

Rejection under 35 U.S.C. §112, second paragraph

Claims 14-25, 27, 29, 38-61, 65 and 66 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants have amended the claims thereby obviating the rejections.

Rejection under 35 U.S.C. §103 (a)

In the January 2, 2003 Office Action, claims 14-25, 27, 29, 38-61 65 and 66 were rejected under 35 U.S.C. §103 (a) as being unpatentable over Donnelly, et al. and Johnson in further view of Whittle, et al. Applicants submit that Donnelly, et al. in combination with Johnson and Whittle, et al. does not render applicants' claimed invention *prima facie* obvious.

The present invention relates to an adeno-associated virus vector comprising a nucleotide sequence encoding a fusion polypeptide. The fusion polypeptide comprises a structural papillomavirus polypeptide and **an early non-transforming** papillomavirus polypeptide having the C-terminus of the structural polypeptide connected to the N-terminus of the early **non-transforming** early polypeptide. The connection between the structural and **non-transforming** early polypeptide is produced by ligating the 3' end of the structural ORF to the 5' end of **early ORF** thereby encoding for the connected fusion polypeptide. The presently claimed invention specifically includes a **non-transforming peptide**, which as described on the top of page 4 of the present specification, refers to the fact that the polypeptide has no transformation ability by nature or through intervention. Transformation is clearly described in the present specification as the conversion of a normal cell into a tumor cell which has the capacity for unlimited proliferation. Thus, the presently claimed invention, by using **only non-transforming early ORFs, eliminates the ability of extracellular DNA from recombining with the cell genome, to prevent giving the cells new genetic properties**.

In the June 13, 2002 Office Action, the Office stated that:

> "while Donnelly et al. and Johnston teach the expression of multivalent antigens of both early and late ORFs of papillomavirus, neither reference provides the specific teaching to combine the various proteins, or fragments thereof, into one fusion protein comprising both an early ORF and a late ORF. Lacking the specific teaching to combine the proteins into one fusion protein, the teachings of Donnelly et al. and Johnson do not make obvious the instantly claimed invention, and the rejection is withdrawn."

By the above statement, it is clear that the Office recognizes that the combination of Donnelly, et al. and Johnson does not establish a *prima facie* case of obviousness. However, the Office mistakenly believes that the combination is only lacking in the teaching of fusion proteins and has

failed to address the fact that **Donnelly et al. teaches away from using the viral vectors of Johnson.**

Johnson relates to a eukaryotic expression system comprising recombinant Adeno-Associated Virus (AVV) vectors that are used in combination with a helper virus for transfection of cells. Viral vectors, especially AAV, cause integration of the AAV genome and any foreign DNA material included in the AAV genome into the chromosomic material of the transfected cell. Because of this integration directly into the DNA of the cell, AAV exhibits long-term gene expression *in vivo*. Thus, genes transfected by the AAV, whether detrimental to the subject or not, are expressed by the transfected cell.

According to the Office, as stated at page 4 in the June 13, 2002 Office Action, Donnelly, et al. teaches that "generally any appropriate vector can be used for delivery of papillomavirus ORF sequences." Applicants vigorously disagree because Donnelly, et al. expressly teaches the use of **artificially engineered plasmids.** Clearly, Donnelly, et al **teaches away** from the use of viral vectors, such as those described by Johnson to infect cells, because as stated at page 4, in the first full paragraph of the Donnelly, et al. reference:

> "Retroviral vectors have restriction on the size and structure of polypeptides that can be expressed as fusion proteins while maintaining the ability of the recombinant virus to replicate, and the effectiveness of vectors such as vaccinia for subsequent immunization may be compromised by immune responses against the vectors themselves. Also viral vectors and modified pathogens have inherent risk that may hinder their use in humans."

Further, Donnelly, et al., expressly states, at the bottom of page 5, that using the described engineered plasmid DNA is **advantageous because no assembly of virus particles is required and no infectious agent is involved.** Thus, Donnelly, et al. explicitly teaches away from using a viral vector such as described by Johnson, mainly for the reason that Johnson teaches that a virus helper is required for transfection (see column 4, line 41). It should be further noted that Donnelly, et al, does not in any way discuss, teach or suggest using only **non-transforming early E6-ORF or E7-ORF** for inclusion in the described **artificially engineered plasmids.** Clearly, the Donnelly, et al. reference is completely silent regarding transformation of transfected cells because

the engineered plasmids described by Donnelly, et al. provide no mechanism for integration of the virus DNA into the cell genome.

Thus understood, applicants request that the Office explain why one skilled in the art, after reading the above quoted sections of Donnelly, et al., would consider using a viral vector which requires the use of a virus helper, such as that taught by Johnson, for transfection of a cell. Clearly, one reading the Johnson reference , which expressly states that a virus helper is required for transfection, and then Donnelly, et al., which teaches the disadvantage of using a virus helper, would not think of combining the two references, with or without Whittle, et al.

To overcome the shortcomings of the Donnelly, et al. and Johnson combination, the Office introduced Whittle, et al. which describes fusion proteins comprising late and early ORFs. However, it is evident that Whittle, et al. does not include any additional teachings that in combination with Donnelly, et al. and Johnson establish a *prima facie* case of obviousness.

As stated above, the presently claimed invention comprises a fusion polypeptide that combines a late structural ORF or fragments thereof and a **non-transforming** E6-ORF or E7-ORF or fragments thereof for inclusion into an AAV viral vector system.

According to the Office:

> "Whittle, et al. teach that at the time of filing many of the ORFs have been used for the production of HPV vaccines (see columns 1-3), and provide specific and necessary guidance to generate polynucleotide sequences which encode fusion proteins comprising late and early ORFs of papillomavirus."

Applicants insist that Whittle, et al. does not in way describe, teach or suggest the presently claimed invention.

According to MPEP 706.02(j):

> "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to

> one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir 1991)."

Firstly, the Office has not identified any objective or specific teachings or suggestions in the cited references that would motivate one skilled in the art to combine the references. According to the Office, "the test for combining references is not what the individual references themselves suggest, but rather what the combination of disclosures taken as a whole would have suggested to one of ordinary skill in the art" (see page 6 of June 13, 2002 Office Action wherein the Office cited a 1971 decision by the CCPA (*In re McLaughlin*, 170 USPQ 209)). Applicants take issue with the cited case law because the cited references in McLaughlin did not present conflicting construction, such as in the present instance. If the Office insists that the references must be taken as a whole then the fact that Donnelly, et al. and Whittle, et al. both teach away from using the viral vector system of Johnson must also be considered. Clearly, Donnelly, et al. teaches away from using viral vectors, and thus one skilled in the art would not consider combining Donnelly, et al. with Johnson, with or without Whittle, et al. Furthermore, Whittle, et al teaches away from using eukaryotic expression systems because of the low yield and instead prefers the use of a prokaryotic expression system. Thus, one would not consider combining the eukaryotic expression system of Johnson with the teaching of Whittle, et al., with or without Donnelly, et al.

The Office should be aware that the Federal Circuit recently addressed the question whether there is a reason to combine references and what is required by the examiner to show a suggestion to combine references and stated: (See *In re Lee*, 61 USPQ3d 1430, 1433 (Fed. Cir. 2002))

> "'The factual inquiry whether to combine references must be thorough and searching.' *Id.* It must be based on **objective evidence of record**. This precedent has been reinforced in myriad decisions, and **cannot** be dispensed with. *See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124-25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000) ("a showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential component of an obviousness holding'") (quoting *C.R. Bard, Inc., v. M3 Systems, Inc.*, 157 F.3d 1340, 1352, 48 USPQ2d 1225,1232 (Fed. Cir. 1998)); *In re*

> *Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) ("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-**based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references**."); *In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (there must be some motivation, **suggestion,** or teaching of **the desirability** of making the specific combination that was made by the applicant); *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988) ("'teachings of references can be combined *only* if there is some suggestion or incentive to do so.'") (emphasis in original) (quoting *ACS Hosp. Sys., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)).
>
> **The need for specificity pervades this authority**. *See, e.g., In re Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected **these components for combination in the manner claimed**"); *In re Rouffet*, 149 F.3d 1350, 1359, 47 USPQ2d 1453, 1459 (Fed. Cir. 1998) ("even when the level of skill in the art is high, the Board must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination.
>
> In other words, the Board must explain the reasons one of ordinary skill in the art would have been motivated to select the references and to combine them to render the claimed invention obvious."); *In re Fritch*, 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992) (the examiner can satisfy the burden of showing obviousness of the combination "**only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references**")." (Emphasis added)

Reading the above quote, it is apparent that the Federal Circuit in 2002 has raised the bar that the Office must meet to show a suggestion or motivation to combine references to establish a *prima facie* case of obviousness, relative to the 1971 case cited by the Office. Applicants submit that the Office has not met the current standard set forth by the Federal Circuit to show a suggestion or motivation to combine the cited references.

Secondly, in light of the fact that both Donnelly, et al. and Whittle, et al. teach away from using the eukaryotic viral vector system of Johnson, there is no reasonable expectation of success. Not one of the fusion proteins described in Whittle, et al. has been introduced into a eukaryotic expression system because Whittle, et al. expressly states that a eukaryotic system generates a low expression level and teaches away from uses an eukaryotic expression system. Accordingly, there is no suggestion or teaching for the combination proposed by the Office.

Thirdly, even if all the references were combinable, which they are not, the combination still does not in any way disclose, teach or suggest each and every limitation of the presently claimed

invention. Specifically, neither Donnelly, et al. nor Whittle, et al., either alone or with Johnson teach or suggest a **fused polypeptide** comprising a structural papillomavirus polypeptide encoded by an open reading frame selected from the group consisting of L1-ORF, L2-ORF and fragments of any of the foregoing ORFs; and a **non-transforming** early E6-ORF, E7-ORF and fragments of any of the foregoing ORFs. Donnelly, et al. is completely silent relating to the inclusion of **non-transforming** E6-ORF and E7-ORF in the described engineered plasmids. Of course, Donnelly, et al. **does not use viral vectors, as discussed above, and thus integration of the early transforming genes into the cell genome is not recognized as a problem.** Likewise, Whittle, et al. does not in any way, disclose, teach or suggest using non-transforming E6-ORF or E7-ORF genes for inclusion in the prokaryotic expression system described in Whittle, et al. Thus, the proposed combination by the Office, does not meet **all** the requirements set forth in the MPEP, as required to establish a *prima facie* case of obviousness.

In light of the above discussion and the fact that (1) there is no motivation, suggestion or teaching to combine the references; (2) each and every recited limitation of applicants' claimed invention is not disclosed or suggested in the cited references; and (3) even if the references were combinable, which they are not, Donnelly, et al. and Whittle, et al. teach away from use of a viral vector such as that disclosed in the Johnson reference; it is clear that the cited combination fails to establish a *prima facie* case of obviousness of applicants' claims as herein amended.

Claims 16, 18, 20 and 50 were rejected under 35 U.S.C. §103 (a) as being unpatentable over Donnelly, et al., Johnson and Whittle, et al. in further view of Gissmann, et al. Regardless, of the teachings of Gissmann, et al., applicants respectfully submit that the defects in the alleged *prima facie* case over Donnelly, et al., Johnson and Whittle, et al. are not cured by the addition of Gissmann, et al.

The Office must view Gissmann, et al. in its entirety and if properly viewed, the cited reference in combination with the primary and secondary references still does not teach or suggest all the claimed limitations of the present invention. Gissmann, et al. discloses fused polypeptides wherein a portion of a viral structural proteins of HPV, whether L1 or L2, is deleted. In the deleted area of the sequence another sequence is inserted. This is in sharp contrast to Donnelly, et

al. that expressly states that maintaining the conserved portions of the papilloma viruses, such as structural portions L1 and L2, is important to provide protection against subsequent challenges by different types of papilloma viruses. Donnelly, et al. maintains the integrity of the structural proteins for the specific reason of providing extended protection, even if a subsequent attack occurs by another virus strain. Clearly, mutating the L1 or L2 structural gene is precluded by Donnelly, et al. and as such the Gissmann, et al and Donnelly, et al. references are not combinable. More important, if the mutation of L1 and L2 as taught by Gissmann, et al. is introduced into the DNA constructs of Donnelly, et al. the intended purpose of maintaining a highly conserved structural protein in the Donnelly, et al. reference is destroyed. Thus, there is no motivation to combine the cited references and the Office has not provided any teachings or suggestions sufficient to provide one skill in the art the motivation to make the proposed modifications needed to arrive at applicants' claimed invention.

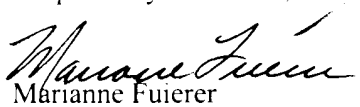
Claim 61 was rejected under 35 U.S.C. §103 (a) as being unpatentable over Donnelly, et al., Johnson and Whittle, et al. in further view of Stanley, et al. Regardless of the teachings of Stanley, et al. applicants respectfully submit that the defects in the alleged prima facie case over Donnelly, et al., Johnson and Whittle, et al. are not cured by the addition of Stanley, et al. Thus, for the reasons set forth above, this rejection also is improper.

In light of the foregoing observations and the clarifying amendments to the claims, applicants submit that the cited references fail to suggest the subject matter of the rejected claims. Reconsideration and withdrawal of the rejections is respectfully requested.

Conclusion

Applicants have satisfied the requirements for patentability. All pending claims are free of the art and fully comply with the requirements of 35 U.S.C. §112. It therefore is requested that Examiner Woitach reconsider the patentability of claims 14-61, 65-66, in light of the distinguishing remarks herein and withdraw all rejections, thereby placing the application in condition for allowance. Notice of the same is earnestly solicited. In the event that any issues remain, Examiner Andres is requested to contact the undersigned attorney at (919) 419-9350 to resolve same.

Respectfully submitted,



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APPENDIX A

THE ENCYCLOPEDIA OF Molecular Biology

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full transforming potential, which interacts with the tumour suppressor protein p53 [5].

Polyoma virus LT shares some functions with its SV40 counterpart. It probably has a similar role in replication; DNA binding and ATP-dependent helicase activity have been demonstrated, and it is also active in transcriptional activation/repression assays. It is less effective, however, in transformation assays and is capable only of promoting proliferation, or IMMORTALIZATION of primary tissue culture cells. Interaction with p105Rb has been shown, but no interaction with p53 has yet been demonstrated.

MT. Mouse and hamster polyomaviruses, but not SV40-type viruses, encode a 55K major early function. The hamster species is relatively poorly characterized, but mouse polyoma virus MT has been much studied. It is responsible for cellular alterations which lead to transformation of proliferating cells to a tumorigenic phenotype. MT is located in higher order structures in the cytoplasm, predominantly membrane and cytoskeletal in nature. In particular, a membrane-bound subpopulation associated with plasma membranes, essential to transformation, interacts with and activates the product of the cellular proto-oncogene *c-src* (*see* ONCOGENES). This complex is also directly associated with the 81K component of a phosphatidylinositol-3 kinase enzyme, which may also be found in association with activated platelet-derived growth factor receptor (*see* GROWTH FACTORS: GROWTH FACTOR RECEPTORS). In addition, MT interacts with the 60K, regulatory (A), and the 35K, catalytic (C), subunits of the cellular protein phosphatase 2A complex. The function of this interaction in transformation by MT is unknown.

The relevance of these interactions to the growth of the virus is similarly unclear. MT is necessary for lytic growth: mutations in the MT/ST unique region result in viruses that grow poorly. There is some correlation between this loss of function and correct post-translational modification of the major capsid protein, VP1, which results in a block in virion assembly.

ST. The small T gene product (23K in size) shares common sequence with the other early antigens of the polyomaviruses for most of its length; in mouse polyoma virus only the C-terminal four amino acids are unique to ST. It is dispensable for achieving most viral functions, but it appears to potentiate the actions of the other early antigens and may have a role in tumour formation *in vivo*. STs from both SV40 and polyoma virus interact with the A and C subunits of protein phosphatase 2A, which localizes the site of this interaction to a cysteine-repeat region common to polyoma virus MT and ST and SV40 ST.

The late region

This region encodes the three structural proteins of the virion. The major coat protein, VP1 (45K), is expressed at late times in the viral life cycle and is initially located in the nucleus. Later, it can be found in the cytoplasm of infected cells as well. In mouse polyoma virus it is modified post-translationally by phosphorylation and acetylation and has six isoelectric forms. It can spontaneously organize, *in vitro*, into aggregates of five molecules known as capsomeres, which, under the appropriate buffer conditions, can form capsids. Despite the capsomers being arranged in the capsid with axes of five- and sixfold symmetry (Fig. P3), only pentamers of VP1 are found.

The role of the minor coat proteins in the virion structure is less well defined. X RAY CRYSTALLOGRAPHIC data have revealed that below the outer layer of VP1 capsomeres lies an electron-dense layer, probably composed of VP2 and 3 (35 and 23K, respectively). VP3 is entirely encoded within VP2 and so its contribution to viral infectivity is difficult to assess, although it is thought to be necessary for efficient viral reproduction. Both proteins accumulate in the nucleus and may be involved in transporting VP1 to this site. VP2 is modified post-translationally with myristic acid and loss of this modification reduces, but does not abolish, infectivity.

Papillomaviruses

It has not been possible to develop an *in vitro* lytic system for the study of papillomaviruses: virus and viral transcripts can only be obtained in very low amounts from infected epithelial tissues or papillomas. However, mRNAs isolated from these sources reveal overlapping and spliced transcripts, and promoters and POLYADENYLATION sites utilized vary, depending on the source and open reading frame (ORF) transcribed. Expression of the viral genes is tightly restricted by the level of differentiation of the cell in the epidermis. With the progression from undifferentiated basal layer cells to differentiated squamous cells, viral gene expression switches from early to late. Viral functions have mostly been analysed by cloning the ORFs into suitable vectors and expressing them in tissue culture. Association of some types of human papillomaviruses (HPVs) with cancerous conditions has stimulated a wealth of data on the functions encoded by these

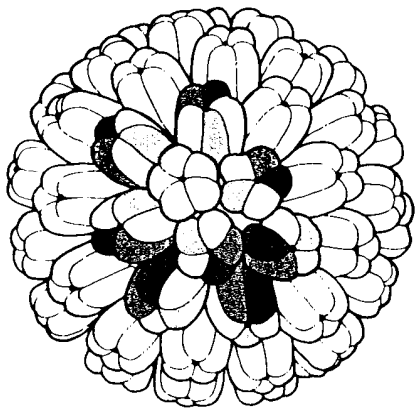

Fig. P3 The packing arrangement of subunits in the papovavirus SV40. The crystal structure of SV40 revealed a new type of packing in which 72 pentamers of virion protein VP1 are arranged in a pattern of interlocking pentagons and hexagons. Twelve 5-coordinated pentamers lie on the 5-fold rotation axes of the icosahedron, each surrounded by five pentamers. Sixty 6-coordinated pentamers do not lie on any symmetry axes and each is surrounded by six pentamers. Based on [7].

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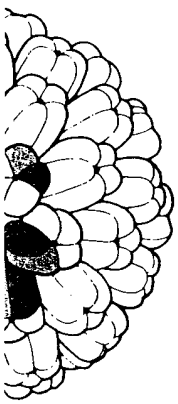
in the virion structure is less HIC data have revealed that meres lies an electron-dense nd 3 (35 and 23K, respec- n VP2 and so its contribution s, although it is thought to be tion. Both proteins accumu- olved in transporting VP1 to dationally with myristic acid ices, but does not abolish,

n *in vitro* lytic system for the viral transcripts can only be infected epithelial tissues or ed from these sources reveal , and promoters and POLY- pending on the source and ped. Expression of the viral l of differentiation of the cell ession from undifferentiated squamous cells, viral gene late. Viral functions have e ORFs into suitable vectors e. Association of some types ith cancerous conditions has functions encoded by these



its in the papovavirus SV40. The pe of packing in which 72 ed in a pattern of interlocking nated pentamers lie on the 5-fold ounded by five pentamers. Sixty symmetry axes and each is

viruses. However, bovine papillomavirus, type 1 (BPV-1) being more readily obtained than other members of the family, is commonly used as a prototype for studying viral functions. As with polyomaviruses, the genome has been divided into early and late regions (Fig. P2), although, unlike polyomaviruses, transcription of both regions occurs from a single strand.

Early region

The region containing early ORFs has been defined as the fragment of the genome (69% of the total) necessary for conferring transformation on cells in an *in vitro* assay. This region contains eight ORFs, five of which (E1, E2, E4, E6, and E7) appear to be found in other papillomavirus genomes, suggesting that these are essential functions.

E1. The E1 ORF gene is involved in episomal (extrachromosomal) replication of the viral genome. It bears some homology with SV40 LT in the regions involved in ATPase activity and nucleotide binding and its product can bind DNA, either alone, or complexed to E2 gene products.

E2. These polypeptides have the properties of transcriptional regulators [6]. They bind as dimers to the palindromic DNA sequence ACCGNNNNCGGT, which is found in several copies in the noncoding region of the virus. Simultaneous binding of E2 dimers to several of these sites results in a high level of transcriptional activation. There is also evidence for cooperation of E2 with cellular transcription factors such as AP1. Active E2 seems to repress promotor/enhancer activity for the ORFs E6 and E7. E2 is often found to be defective in material derived from HPV tumours.

E6, E7. The major proteins encoded by papillomaviruses that are relevant to transformation are transcribed from the E6 and E7 ORFs. These polypeptides are capable of transforming primary cells in a classical focus formation assay. The E6 protein, from HPV 16 and 18, can promote the breakdown of the tumour suppressor protein p53, in an ATP-dependent manner and utilizing the ubiquitin degradation pathway (*see* PROTEIN DEGRADATION). E7, which cooperates with the oncogene *ras* to transform primary cells, has been shown to interact with the underphosphorylated form of the p105Rb tumour suppressor gene. This interaction probably occurs through the N-terminal 37 amino acids, which share some homology with domains 1 and 2 of adenovirus E1A, also known to be involved with p105Rb binding. Therefore, transformation of cells by E6 and E7 may occur by a similar route to that of the DNA tumour viruses – adenovirus and SV40. E6 and E7 may also induce host chromosomal alterations.

E5. A further contribution to transformation, in some papillomaviruses, may come from the product of the E5 ORF. Studies on the 44 amino acid product of deer or bovine papillomavirus E5 ORFs have revealed a potential for the gene in inducing growth, cellular transformation, DNA synthesis, and activation of the platelet-derived growth factor receptor. Various mechanisms for the latter have been proposed, including direct binding to the receptor. Some homology with the β chain of platelet-derived growth factor (*see* GROWTH FACTORS) has been noted. The E5 protein has been isolated in association with a 16K protein thought to be important in cellular compartments in which processing of growth factor receptors occurs.

Late region

The late region has two large ORFs capable of coding for proteins of 55K (L1) and 50K (L2). L1 encodes the major structural capsid protein. The role of the minor capsid protein, L2, is not known.

N.S. KRAUZEWICZ

See also: ANIMAL VIRUSES; ANIMAL VIRUS DISEASE; EUKARYOTIC GENE EXPRESSION.

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7 Liddington, R.C. et al. (1991) Structure of simian virus 40 at 3.8-Å resolution. *Nature* 354, 278–284.

paracentric inversion *See*: CHROMOSOME ABERRATIONS.

paracrine Term applied to the action of local chemical mediators which have their effects very close to the cells that secrete their

paralogous *See*: HOMOLOGY.

paramylon A storage β1,3 glucan of the unicellular alga *Euglena gracilis*, formed by transfer from UDP-Glc, using a paramylo synthetase that is membrane-associated and solubilized by so dium deoxycholate. There is evidence for a GLYCOPROTEIN prime and the synthetase closely resembles CALLOSE synthetase in being stimulated by oligosaccharides of β1,3 and β1,4 glucans.

Paramyxoviridae Family of enveloped RNA ANIMAL VIRUSES com prising three genera – paramyxoviruses, morbiliviruses (measle canine distemper), and pneumoviruses (respiratory syncyti virus, RSV). Virus particles are pleomorphic and ~150 nm diameter, with surface spikes. The single-stranded RNA genom (M_r 5×10^6–7×10^6) is usually (–) sense, but some particl contain (+)-sense genomes. *See*: ANIMAL VIRUS DISEASES; ANIM. VIRUSES.

paranemin An INTERMEDIATE FILAMENT protein.

paraquat One of the low-potential (E_m ~ –400 mV) viologen dy

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HUMAN PAPILLOMAVIRUS E6 **AND** E7 - **PROTEINS WHICH DEREGULATE THE CELL-CYCLE**

TOMMASINO M, CRAWFORD L

BIOESSAYS

17 (6): 509-518 JUN 1995

Document type: Review **Language:** English **Cited References:** 62 **Times Cited:** 77

Abstract:

Numerous clinical, epidemiological and molecular findings link some types of Human Papillomaviruses (**HPV**) with cancer of the genital tract, They share a common pathway of **transformation** with a number of DNA tumour viruses, such as Adenovirus and SV40. Although all these viruses are termed 'DNA tumour viruses' and have similar in vitro **transforming** activities, Human **Papillomavirus** is the only one so far clearly involved in human cancer. Extensive studies on **HPV E6** and **E7** proteins have demonstrated their involvement in malignant **transformation**. **E6** and **E7** bind the products of tumour suppressor genes, p53 and Rb1, respectively, modifying or inactivating their normal functions, The Rb1 and p53 genes are deleted or mutated in several cancers and both proteins regulate the transcription of genes involved in cell cycle progression control. The **E6**/p53 and **E7**/Rb1 interactions result in a deregulation of the cell cycle with loss of control of crucial cellular events, such as DNA replication, DNA repair and apoptosis.

KeyWords Plus:

RETINOBLASTOMA GENE-PRODUCT, TYPE-16 **E7**, HUMAN KERATINOCYTES, COMPLEX-FORMATION, ZINC-BINDING, EJ-RAS, P53, **TRANSFORMATION**, ASSOCIATION, ONCOPROTEIN

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THE EXPRESSION LEVELS OF THE HUMAN PAPILLOMAVIRUS TYPE-16 E7 CORRELATE WITH ITS TRANSFORMING POTENTIAL

LIU ZJ, GHALI J, OSTROW RS, FARAS AJ

VIROLOGY
207 (1): 260-270 FEB 20 1995

Document type: Note **Language:** English **Cited References:** 66 **Times Cited:** 11

Abstract:
The **transforming** potential of the human **papillomavirus** (**HPV**) type 16 has been defined largely in the **E7**, **E6**, and E5 oncoproteins, with the major **transforming** capability residing in the **E7** gene. In this paper, we found that in cooperation with the activated res the HPV16 **E7** gene when expressed in a retroviral vector could fully **transform** baby rat kidney (BRK) cells in transfections, whereas the same construct could only immortalize the BRK cells following retroviral infection. This inability to **transform** correlated with the low levels of **E7** gene RNA expression in the viral infected cells, which harbor a lower number of copies of the **E7** gene constructs. Cotransfection of the expression vector FV2E7, which gives high levels of **E7** gene expression, and activated ras lead to rapid and efficient morphological **transformation** of BRK cells which grew easily in soft agar and induced large tumors in athymic nude mice. In contrast, cotransfections of the expression vector FV1E7, which gives lower levels of 67 gene expression, produced much lower numbers of **transformed** colonies which took longer to form, showed a retarded growth on soft agar, and induced smaller tumors in nude mice. Under these conditions, colonies of immortalized, but morphologically untransformed cells formed in large numbers. These results indicate that the **transforming** potential is directly correlated to the expression levels of the oncoprotein and that a threshold level of the **E7** oncoprotein may be required before the cells can be fully **transformed**. This supports the hypothesis that the **transformation** processes include at least two separate and continuous steps which first lead to immortalization and then to metastasis, in agreement with the clinical progression of genital tumors from benign to malignancy. Such a progression may involve enhanced expression of the oncoproteins. (C) 1995 Academic Press, Inc.

KeyWords Plus:
CARCINOMA CELL-LINES, OPEN READING FRAME, CERVICAL-CARCINOMA, ONCOGENIC **TRANSFORMATION**, HUMAN KERATINOCYTES, ACTIVATED RAS, GENE-PRODUCTS, **E6** PROTEIN, DNA, IMMORTALIZATION

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